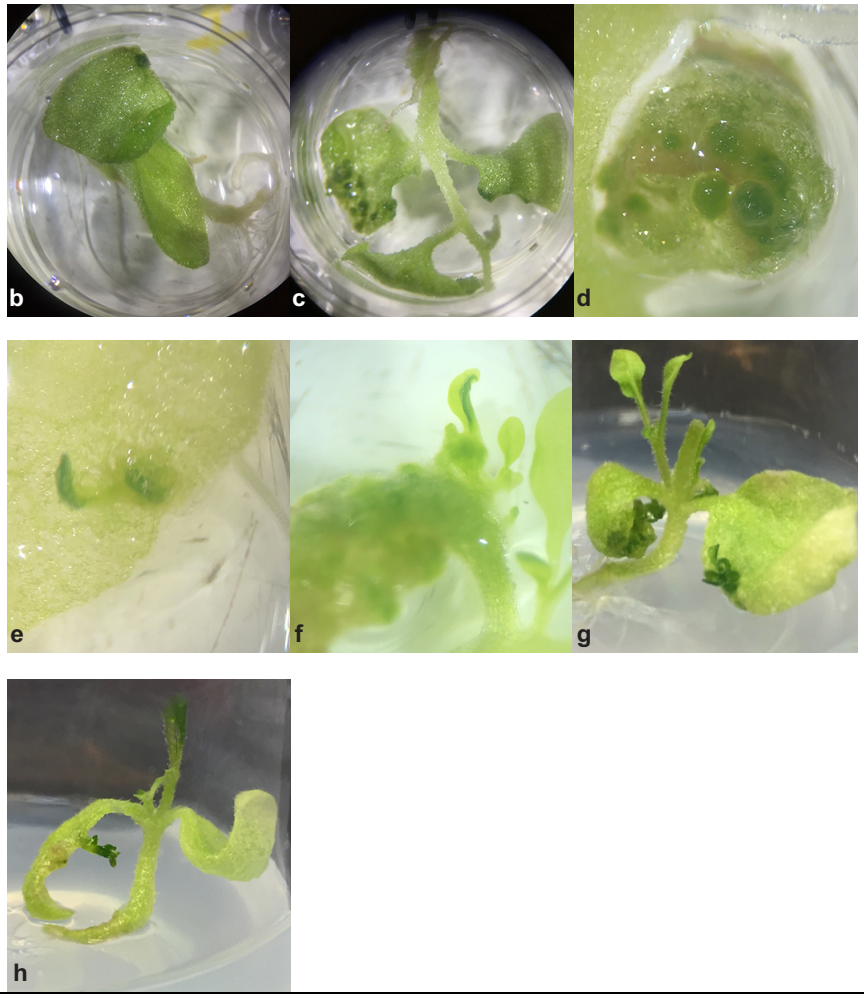
a

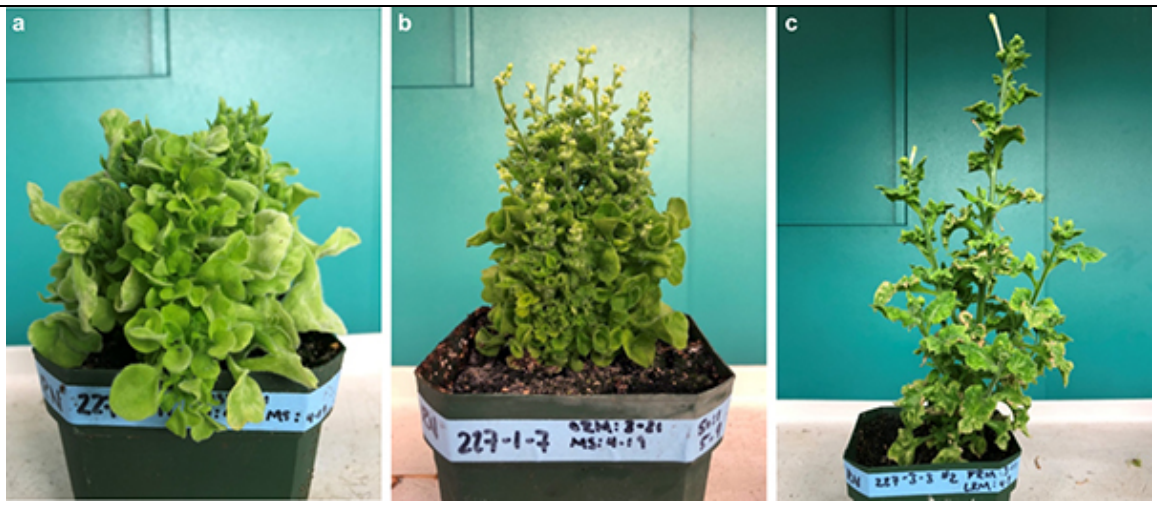
Treatment	Total seedlings	Luciferase positive	Seedlings forming growths	% of starting seedlings
No regulator	24	0	0	0
nos:Wus2 & CmYLCV:*STM*	48	38	10	21
nos:Wus2 & 35S:*STM*	48	35	11	23
nos:Wus2 & At*UBQ10:STM*	48	40	15	31



Supplementary Figure 1

Generating *de novo* meristems on seedlings.

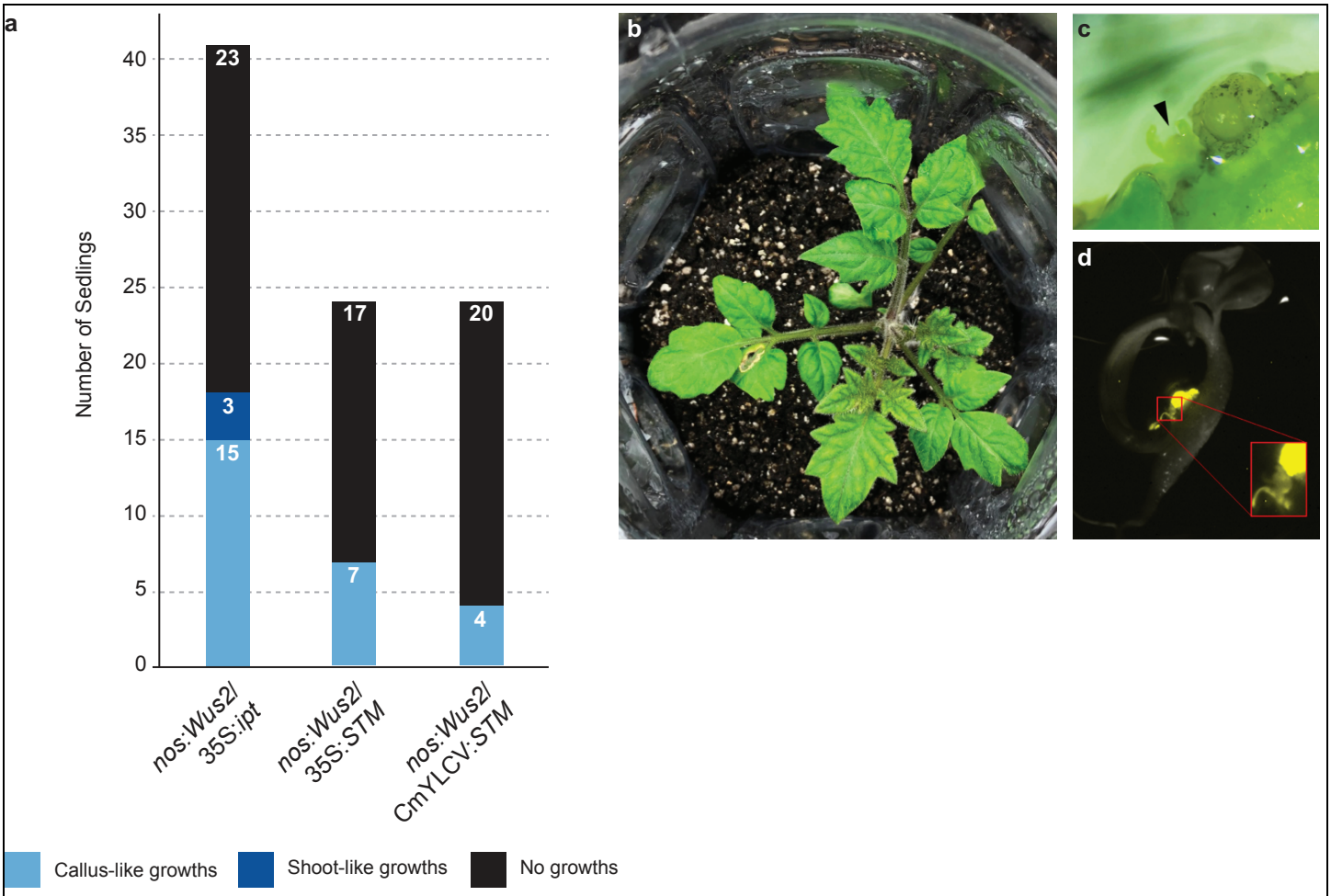
a, *Wus2* and *STM* induced growths on 21-31% of treated seedlings. Little difference in frequency was observed between different promoters used to express *STM*, likely because all drive high expression in *N. benthamiana*. Approximately 12 days after initiating Fast-TrACC, callus-like growths formed, varying from one **b** to many **c-d** per seedling. Over the next 10-14 days, the growths expanded in size and either remained in an undifferentiated state or began to differentiate **e-f.** Shoot-like structures then formed **g-h**, which can be excised and moved to rooting medium. The growths depicted are representative of those observed in three independent experiments.



Supplementary Figure 2

Plants with developmental abnormalities.

Some transgenic plants displayed developmental abnormalities. Shown here are plants derived from treating seedlings with *Wus2* and *STM*. Phenotypes include **a** large numbers of leaves, **b** excess branching and **c** misshapen leaves. The three plants depicted are representative of plants derived from the 36 growths obtained in the experiment described in Supplementary Figure 1.

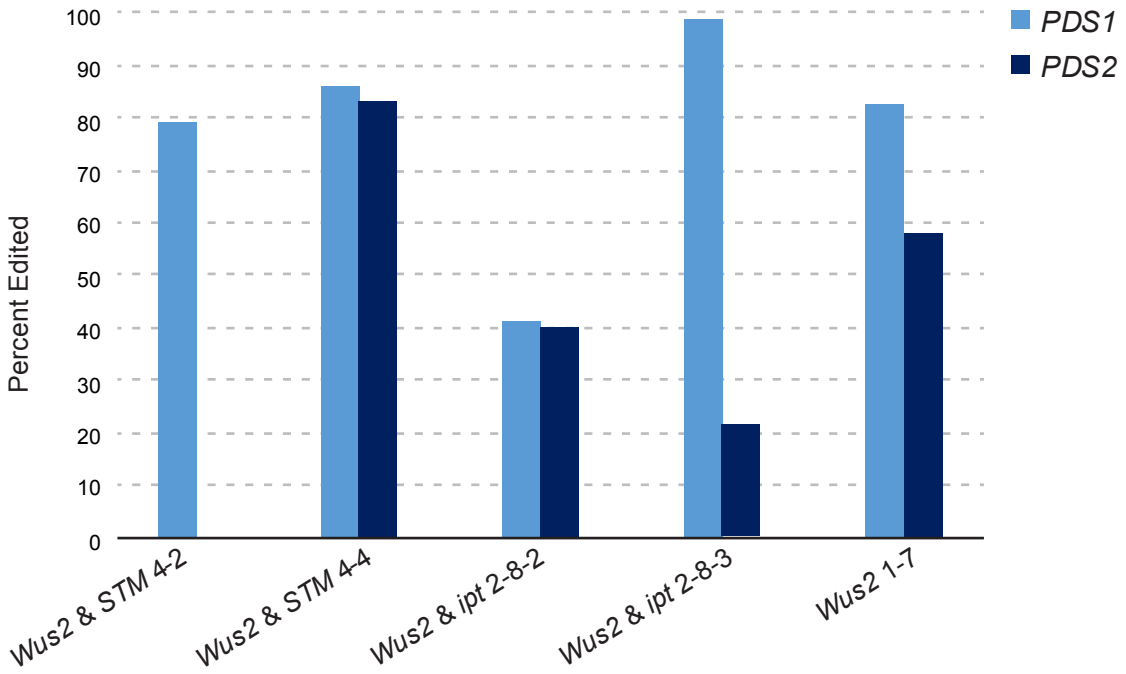


Supplementary Figure 3

Inducing *de novo* meristems in tomato using Fast-TrACC.

a, To induce *de novo* meristems in tomato, three combinations of DRs (*nos:Wus2 & 35S:STM*, *nos:Wus2 & CmYLCV:STM*, *nos:Wus2 & 35S:ipt*) were selected because they effectively induced meristems in *N. benthamiana*. (Supplementary Table 1). For both combinations of *Wus2* & *STM*, no shoot-like growths formed. In contrast, *Wus2* & *ipt* promoted shoot-like growths, which **b**, ultimately formed fully rooted plants. When a luciferase reporter was delivered with the DRs, meristems were induced (**c**, black arrow) that were positive for luciferase activity (**d**, red box and enlarged image shown as inset).

a



b

Edited Plant	No Edit	1bp Insertion	1bp Deletion	2-5bp Insertion	2-5bp Deletion	5-10bp Insertion	5-10bp Deletion
4-2	18.3 / _	0 / _	0 / _	0 / _	41.3 / _	0 / _	40.4 / _
4-4	18.3 / 27.5	79.7 / 61.2	0 / 0	0 / 4.9	0 / 1.2	0 / 5.2	2 / 0
2-8-2	63 / 69.2	26.8 / 26.4	0 / 0	0 / 0	1.9 / 0	4.2 / 4.4	4.1 / 0
2-8-3	2.4 / 85	96.5 / 7.9	0.6 / 0	0.5 / 3.2	0 / 1.2	0 / 1.4	0 / 1.3
1-7	16.1 / 43.9	41 / 4.1	0 / 45.1	0 / 0	41.8 / 1.5	0 / 1.5	1.1 / 3.9

Supplementary Figure 4

Gene edits are transmitted vertically by plants derived from *de novo* meristems induced by Fast-TrACC.

a, Five gene edited plants were produced by treating seedlings with *Wus2* & *ipt*, *Wus2* & *STM* or *Wus2* alone. The type and amount of editing was determined by Sanger sequencing and TIDE analysis (see Methods). The predicted editing percentage for each *PDS* gene is shown. **b**, The types of edits predicted by TIDE are shown as percentages of total editing. Any percentage below 5% was deemed below the limit of detection by TIDE. The first number in the column is indicative of the percentage of that edit in *PDS1*; the second number is indicative of editing in *PDS2*. Because of a deletion in 4-2, the sequence was not able to be properly aligned and thus no percentages are given for *PDS2*. See also Supplementary Data Set for DNA sequences of all mutants recovered.

Supplementary Figure 5

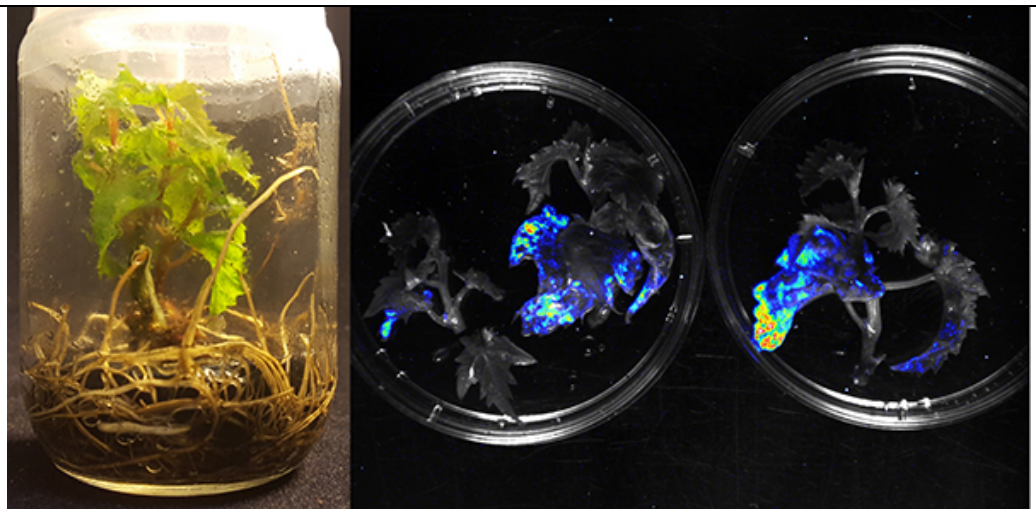
Heritability of *N. benthamiana PDS* editing events created by Fast-TrACC.

a, An example of the phenotypes of seedlings derived from flowers F4 and F5 of plant 1-7 (see also Supplementary Table 4). The fully green seedlings are healthy; the fully white seedlings do not develop after germination; the chimeric plants are stunted. **b**, Chimeric seedlings retain the transgene that carries the sgRNA expression cassette. DNA from seedlings was PCR-amplified using primers that specifically amplify the transgene. Chimerism is likely due to continued mutagenesis at *PDS1*. The image in panel **a** is representative of experiments performed once with seeds derived from both flowers F4 and F6. The experiment in panel **b** was performed once.

Supplementary Figure 6

Representative images of phenotypes observed for newly formed tissues at developmental regulator delivery sites.

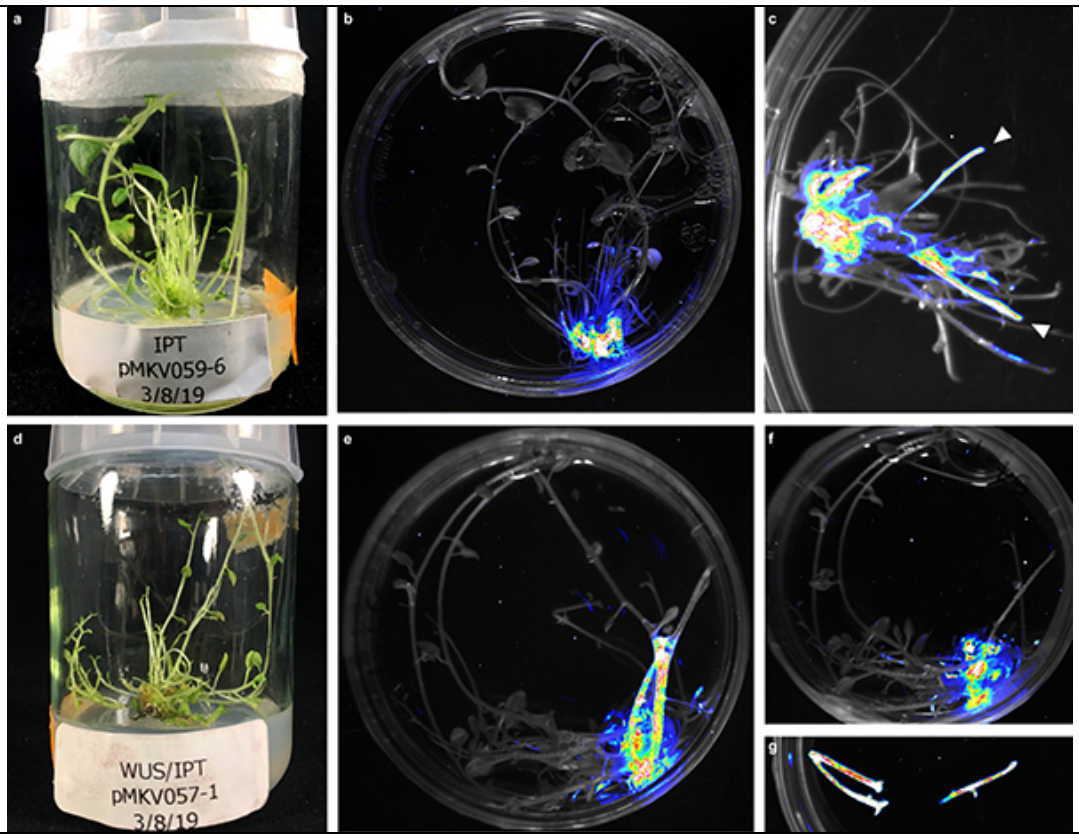
a-d, Many of the phenotypes suggest improper meristem patterning, likely due to persistent expression of developmental regulators. **b**, Some shoots and leaf tissues are white, suggesting biallelic mutations in both *PDS* homologs; **c**, other tissues show a mixture of both green and white phenotypes. **d**, When floral tissues formed on morphologically abnormal tissues, they are abnormal and typically infertile. **e**, Some shoots appear phenotypically normal. The shoot images are representative of the nine white shoots and 30 distorted shoots obtained in the experiment described in Fig. 5c.



Supplementary Figure 7

Delivery of developmental regulators to grape plants induces phenotypically normal transgenic shoots.

The left image is an example of a grape plant 40 days after inoculation with five *A. tumefaciens* strains each carrying a T-DNA vector that expresses a single developmental regulator (pMM230-234). Newly formed shoots were generated at inoculation sites. The image on the right shows shoots that were removed from the plant on the left, exposed to luciferin and imaged for bioluminescence. Bioluminescent tissues indicate newly formed, transgenic shoots that express the luciferase reporter. Images are from a single experiment performed in grape and represent one of six inoculated plants.



Supplementary Figure 8

Delivery of developmental regulators to potato plants induces abnormal shooting phenotypes and transgenic shoots.

a, Potato plant pMKV059-6 displays abnormal shooting ~100 days post inoculation with an *A. tumefaciens* strain carrying a T-DNA with *ipt* and luciferase expression cassettes. **b**, Luciferase expression is observed in tissues and shoots of plant pMKV059-6. **c**, Fully transgenic shoots (white arrowheads) are more easily seen after trimming away several wild-type shoots. **d**, Potato plant pMKV057-1 displays abnormal shooting ~100 days post inoculation with an *A. tumefaciens* strain carrying a T-DNA with *ipt*, *Wus2* and luciferase expression cassettes. **e**, Luciferase expression is observed in tissues and shoots of plant pMKV057-1. **f**, Plant pMKV057-1was trimmed, leaving some transgenic tissue; **g**, fully transgenic shoots could be isolated. The images are representative of two experiments performed in potato.

Construct	Plant designator	Sequences analyzed	Insertions	Deletions	Indel Frequency	Observed mutations	Seed Produced?
Wus2/*STM*	5-13-3-12	1628	0	821	821 (50.4%)	-1bp & WT	No
Wus2/*ipt*	7-19-2-11 **	1089	0	1072	1072 (98.4%)	-10bp & -29bp	No
Wus2/*ipt*	7-19-2-12 **	2431	0	2399	2399 (98.7%)	-10bp & -29bp	No
Wus2/*ipt*	7-19-2-13 **	528	0	493	493 (93.4%)	-10bp & -29bp	No
All Combo	5-14-1-08	258	0	104	104 (40.3%)	-3bp & WT	Yes
All Combo	1-1-5	1057	111	892	1003 (94.9%)	Chimeric: spectrum of +1bp, -3bp, -1bp or larger	No

*Sequence reads were processed as described in the Methods; WT = wild type sequence
** Shoots derived from the same injection site have the same mutation profile, suggesting they are derived from common, edited progenitor cells.

Targeted PDS2 mutations observed:

```
WT:          GGAATTTGTTATGTTTTGGTAGTAGCGACTC-CATGGGGCATAAGTTTAGAATTCG
1-1-5 #1:    GGAATTTGTTATGTTTTGGTAGTAGCGA----CATGGGGCATAAGTTTAGAATTCG
1-1-5 #2:    GGAATTTGTTATGTTTTGGTAGTAGCGACT--CATGGGGCATAAGTTTAGAATTCG
1-1-5 #3:    GGAATTTGTTATGTTTTGGTAGTAGCGACTCCCATGGGGCATAAGTTTAGAATTCG
1-1-5 #4:    GGAATTTGTTATGTTTTGGTAGTAGCGACTC-CATGGGGCATAAGTTTAGAATTCG
1-1-5 #5:    GGAATTTGTTATGTTTTGGTAGTAGCGA-----ATGGGGCATAAGTTTAGAATTCG
1-1-5 #6:    GGAATTTGTTATGTTTTGGTAGTAG-------CATGGGGCATAAGTTTAGAATTCG
1-1-5 #7:    GGAATTTGTTATGTTTTGGTAGTAGCG----------------------------
1-1-5 #8:    GGAATTTGTTATGTTTTGGT------------ATGGGGCATAAGTTTAGAATTCG
1-1-5 #9:    GGAATTTGTTATGTTTTGGTAGT----------ATGGGGCATAAGTTTAGAATTCG
1-1-5 #10:   GGAATTTGTTATGTTTTGGTAGTAG--------ATGGGGCATAAGTTTAGAATTCG
1-1-5 #11:   GGAATTTGTTATGTTTTGGTAGTA------------GGGCATAAGTTTAGAATTCG
1-1-5 #12:   ---------------------------------ATGGGGCATAAGTTTAGAATTCG
1-1-5 #13:   ----------------------------------------------------------
```

Supplementary Figure 9

Evidence for gene editing in plants derived from the experiment described in Fig. 4b.

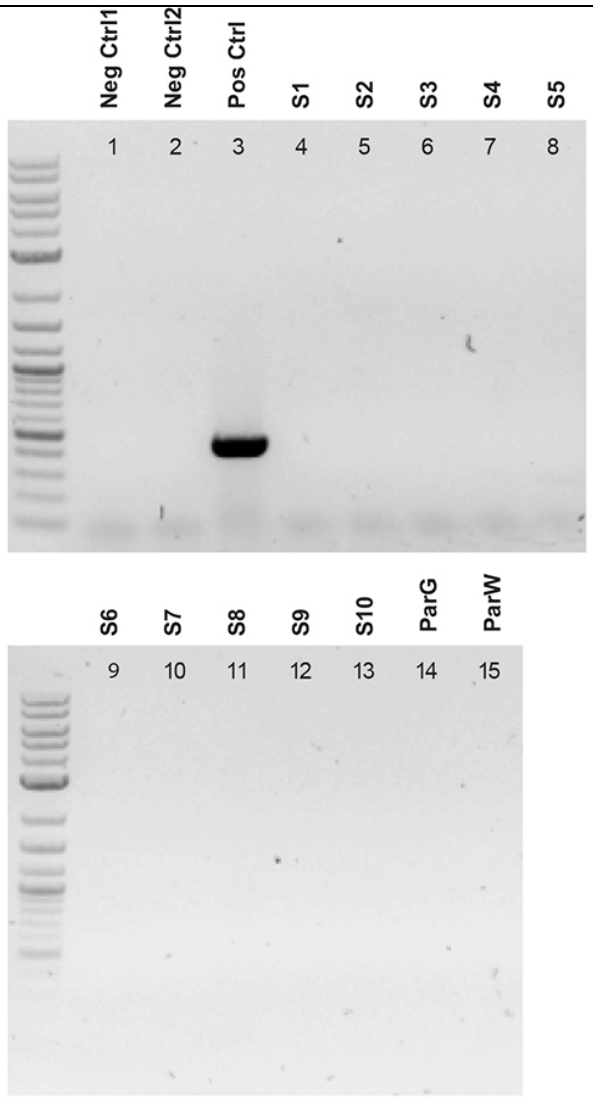
Plants were treated with constructs that included a single vector containing *Wus2* and *STM* (*Wus2*/*STM*), a single vector containing *Wus2* and *ipt* (*Wus2*/*ipt*), or a combination of co-inoculated vectors each containing a single DR (All Combo, see Methods). Derived shoots were given individual designators to facilitate tracking of samples. Purified genomic DNA was amplified for the *PDS2* locus. Amplicons were pooled and submitted for next generation sequencing. 'Sequences analyzed' denotes the observed number of sequences containing the expected forward and reverse barcodes. 'Insertions' denotes the number of sequences observed to have non-specific DNA insertions at the sgRNA target site. 'Deletions' denotes the number of sequences observed to have targeted mutations at the sgRNA target site. 'Indel frequency' denotes the total number of sequences that were observed to have targeted modifications. 'Observed mutations' denotes mutations observed at a frequency >30% of the total. 'Seed produced' denotes those sampled shoots that were identified to have produced seed. With the exception of plant 1-1-5, sequences of the resulting mutations are shown in Fig. 5b. Below the table are the spectrum of mutations recovered for plant 1-1-5.

Sample	Phenotype	*PDS1*	*PDS2*
Parental white tissue	White	+1 (18%) / -1 (34%) -2 (21%) / -3 (16%)	-48 / +1 / -1
Seedling 1	White	+1 (42%) / -2 (49%)	-1 (98%)
Seedling 2	White	+1 (43%) / -2 (48%)	-1 (96%)
Seedling 3	White	+1 (39%) / -2 (51%)	+1 (44%) / -1 (46%)
Seedling 4	White	-2 (95%)	+1 (49%) / -1 (37%)
Seedling 5	White	+1 (46%) / -2 (46%)	+1 (99%)
Seedling 6	White	+1 (51%) / -1 (41%)	+1 (42%) / -1 (45%)
Seedling 7	White	No data	+1 (58%) / -1 (35%)
Seedling 8	White	+1 (55%) / -2 (35%)	+1 (36%) / -1 (35%)
Seedling 9	White	+1 (100%)	+1 (49%) / -1 (42%)
Seedling 10	White	+1 (41%) / -2 (48%)	+1 (98%)

Supplementary Figure 10

Evidence for gene editing in seedlings derived from the white seed pod in Fig. 6.

Observed mutations in white parental tissues and progeny of the white seed pod shown Fig. 6. Genomic DNA was extracted from parental tissue and seedlings and submitted for Sanger sequencing. Sequences were assessed for mutations by TIDE sequence trace analysis (see Methods). All seedlings were white, as indicated by the photo. See also Supplementary Data Set for the DNA sequences all all mutations recovered.

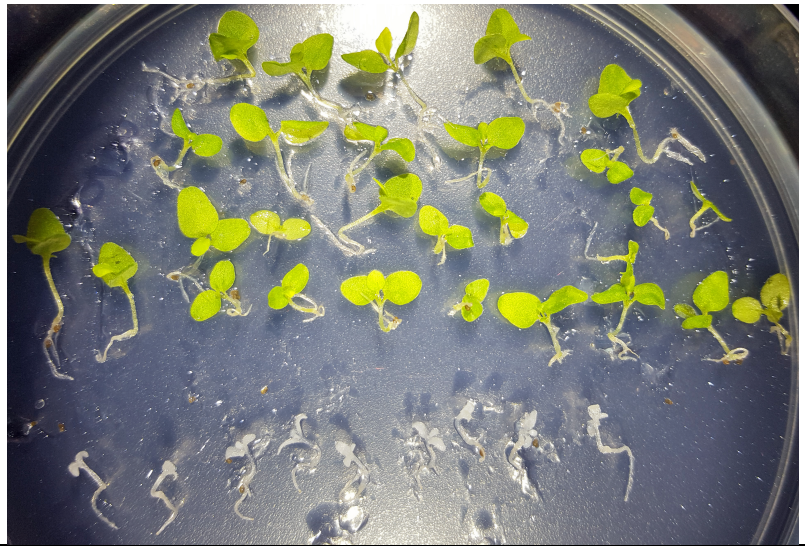


Supplementary Figure 11

Gene-edited seedlings lack integrated T-DNA.

Genomic DNA was extracted from parental white (ParW) and parental green (ParG) tissues of a plant demonstrating targeted editing (Fig. 6) as well as from 10 seedlings derived from the white flower (S1-S10; Extended Data Fig. 10). Genomic DNA was additionally extracted from plants that did not receive the vector (Neg Ctrl 1 and 2) as well as from leaf tissue infiltrated with the target vector (Pos Ctrl). DNA was amplified using primers specific to the U6 promoter present on the T-DNA (expected 448bp). Ladder = New England Biolabs 2log. The experiment was performed once.

Sample	Phenotype	*PDS1*	*PDS2*
Parental green tissue	Green	-1bp (100%)	Heterozygous -48/-1
Seedling 1	Green	-1 (99%)	Heterozygous -48/-1
Seedling 2	Green	-1 (99%)	Homozygous -48
Seedling 3	Green	-1 (100%)	Heterozygous -48/-1
Seedling 4	Green	No data	No data
Seedling 5	Green	-1 (100%)	Heterozygous -48/-1
Seedling 6	Green	-1 (100%)	Heterozygous -48/-1
Seedling 7	Green	-1 (100%)	Homozygous -48
Seedling 8	Green	-1 (98%)	Heterozygous -1/-48
Seedling 9	Green	-1 (100%)	Homozygous -48
Seedling 10	Green	-1 (100%)	Heterozygous -48/-1
Seedling 28	White	-1 (100%)	Homozygous -1
Seedling 29	White	-1 (100%)	Homozygous -1
Seedling 30	White	-1 (100%)	Homozygous -1
Seedling 31	White	-1 (100%)	Homozygous -1
Seedling 32	White	-1 (100%)	Homozygous -1



Supplementary Figure 12

Evidence for gene editing in seedlings derived from the green seed pod in Figure 6.

Observed mutations in green tissues and progeny from the green seed pod in Fig. 6. Genomic DNA was extracted from parental tissue and seedlings and submitted for Sanger sequencing. Sequences were assessed for mutations by TIDE sequence trace analysis (see Methods). The photo shows the wild type and *pds* phenotype of seedlings demonstrating a 3:1 segregation. Note: the 48bp deletion is an in-frame deletion, which appears to retain *PDS* function.